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Monocyte Function Is Normal in Quiescent Psoriasis

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We report an investigation of peripheral blood monocytes from untreated patients with mild, quiescent psoriasis. Possible metabolic changes were monitored by the determination of 3 enzymes representing different pathways of glucose metabolism and 2 lysosomal enzymes. Signal processing was evaluated by the measurement of cyclic AMP levels before and after hormonal stimulation. Luminol-amplified chemiluminescence provided an objective approach to assessing phagocytic capacity. Finally, the pattern of maturation of normal and psoriatic monocytes has been compared during culture in vitro.

Our results were uniformly and wholly negative; we conclude that the concept of an "intrinsic" abnormality of the psoriatic monocyte may be excluded. Possible reasons for discrepancies in the literature are discussed.

During the last decade a number of publications have appeared concerning metabolic or functional aspects of circulating leukocytes in patients with psoriasis. Changes have been reported in various purified or partially purified preparations, including granulocytes, monocytes, lymphocytes, and mixed mononuclear leukocytes (MNL). Unfortunately, the various authors are by no means in agreement in their conclusions; claims have included specific abormalities apparently characteristic of the psoriatic genotype, various "secondary" alterations (i.e., changes paralleling disease activity), and, occasionally, entirely negative findings. The field has recently been the subject of a comprehensive and balanced review [1].

This uncertainty is particularly frustrating in the case of the monocyte/macrophage cell line. It has already been pointed out that an intrinsic fault in the psoriatic monocyte is compatible with the breakdown of epidermal homeostasis which occurs in the skin lesion [2]; it is thus of paramount importance to establish whether such an abnormality really exists. We have therefore undertaken a detailed investigation of this cell in psoriasis. Measurements have been made at various levels, including metabolic (3 enzymes of intermediary metabolism and 2 acid hydrolases), signal processing (cyclic AMP), phagocytic ability (chemiluminescence), and certain parameters of maturation in vitro. Throughout the investigation extreme care has been taken to avoid artifacts arising from environmental circumstances such as hospitalization or therapy, and to eliminate temporal "drift" by the use of paired subjects rather than independent groups. Our findings are presented below.

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Abbreviations:
ACP: acid phosphatase
G-6-PDH: glucose-6-phosphate dehydrogenase
GBSS: Gey's balanced salt solution without phenol red containing 0.2% bovine serum albumin
ICD: isocitrate dehydrogenase
MNL: mononuclear leukocytes
NAG: N-acetylglucosaminidase
PBSA: phosphate-buffered saline containing 0.2% human albumin
PBST: phosphate-buffered saline containing 5 mM $MgCl_2$ and 7.5 mM theophylline
PG: prostaglandin
PHI: phosphohexose isomerase

MATERIALS AND METHODS

Materials

Heparin was obtained from Diosynth BV, Oss, NL, and Ficoll-Paque from Pharmacia, Uppsala, Sweden. All materials for tissue culture were supplied by Gibco Europe BV, Hoofddorp, NL. Luminol and zymosan were purchased from the Sigma Chemical Co, St. Louis, Missouri; enzyme substrates were supplied either by Sigma or Boehringer Mannheim BV, Amsterdam. Diamidino-2-phenylindole was obtained from Serva Feinbiochemica GmbH, Heidelberg, FRG, adrenaline from Centra Chemie BV, Etten-Leur, NL. Reagents for the measurement of cyclic AMP were supplied by New England Nuclear, Doorn, NL. Bovine serum albumin was purchased from Organon BV, Oss, NL and human serum albumin from the Institut Merieux SA, Lyon, France.

All other chemicals were of the highest purity available and were supplied by Merck (Darmstadt, FRG).

Subjects

Most of the patients taking part in this study were drawn from the Association of Psoriatic Patients of the Netherlands (Nederlandse Vereniging voor Psoriasis Patienten); these patients in general had relatively mild plaque type lesions. A few patients were referred from the Dermatology Department of this hospital; this group had more extensive lesions. None of either group had received systemic therapy in the year prior to investigation, and none had received therapy of any kind for at least 2 months. Control subjects were paid volunteers with no personal or family history of psoriasis. Relevant data for the psoriatic and control groups are summarized in Table I; some subjects participated in more than one set of measurements.

Isolation and Identification of Cells

Blood (5–40 ml) was obtained by venepuncture and heparin (10 IU per ml) added as anticoagulant. MNL were isolated by isopycnic centrifugation on Ficoll-Hypaque [3] followed by 3 washings with phosphate-buffered saline containing 0.2% human albumin (PBSA). This preparation was either used directly (chemiluminescence) or further fractionated by counterflow centrifugation [3] (cyclic AMP, enzyme measurements, culture experiments).

Total cell counts were performed using a Coulter counter (model DN) or hemocytometer. Differentiation of cell types utilized either May-Grünwald-Giemsa or nonspecific esterase staining of cytocentrifuge preparations.

Culture Technique

This was described previously. In brief, isolated monocytes were resuspended in RPMI 1640 medium (enriched with 10% autologous serum) at a density of approximately 3×10^5 cells/ml, and 1-ml portions dispensed into Costar Leighton culture tubes (5-cm^2 culture surface). The monocytes were allowed to mature at 37°C for the following 2 weeks.

Enzyme and DNA Measurement

These procedures have already been described in full [4]. Cells were lysed in a solution of bovine serum albumin in water (1 mg per ml) using either one cycle of freeze-thawing followed by sonication (fresh monocytes) or 3 cycles of freeze-thawing (cultured monocytes). After centrifugation, the activities of 5 enzymes were determined using

TABLE I. *Composition of experimental groups; data for controls in parenthesis*

Investigation	No.	M/F	Age	Years psoriasis	Percentage lesions
Enzyme measurements	9 (12)	4/5 (10/2)	42 ± 17 (30 ± 5)	17 ± 16	8 ± 6
Cyclic AMP	9 (9)	4/5 (8/1)	42 ± 16 (29 ± 5)	22 ± 15	10 ± 4
Chemiluminescence	19 (19)	7/12 (13/6)	43 ± 17 (32 ± 11)	20 ± 16	10 ± 13

duplicate 20-μl samples of the appropriately diluted supernatant. In brief, glucose-6-phosphate dehydrogenase (G-6-PDH), phosphohexose isomerase (PHI), and isocitrate dehydrogenase (ICD) were measured by the fluorescence of NADPH released during NADP-linked assays; acid phosphatase (ACP) and N-acetylglucosaminidase (NAG) were determined by the fluorescence of 4-methylumbelliferone released from the appropriate substrates. In all cases reaction rates were shown to be proportional to the concentration of cell lysate at the dilution employed.

The DNA contents of the supernatants were measured by fluorescence of the complex with 4′,6-diamidino-2-phenylindole [5] using a modification described previously [6].

Cyclic AMP Measurements

Freshly-isolated monocytes were resuspended in phosphate-buffered saline containing 5 mM $MgCl_2$ and 7.5 mM theophylline (PBST) at a density of 4×10^6 cells per ml. After 20-min preincubation at 37°C, an aliquot of 100 μl was "flash-heated" (2 min, 100°C) and acidified with 50 μl of 0.1 N HCl. Two additional aliquots of 25 μl were mixed with 75 μl of adrenaline or prostaglandin E_1 (PGE_1) in PBST (final concentrations of 100 μM and 10 μM, respectively). After a 5-min further incubation these were flash-heated and acidified as before. All samples were centrifuged and duplicate 50-μl aliquots of the supernatants taken for assay of cyclic AMP.

Cyclic AMP was measured by radioimmunoassay following acetylation. The reagents employed gave linearity over the range 10–500 fmol per assay. Serial dilution of a single pooled extract plus a parallel "spiked" series indicated a recovery of better than 90% and a reproducibility of ± 15%. Agreement between duplicates was usually better than 10%; samples where this was not achieved were disregarded.

Chemiluminescence

The washed MNL pellet was resuspended in Gey's balanced salt solution without phenol red containing 0.2% bovine serum albumin (GBSS) at a concentration of about 5×10^6 cells per ml. Duplicate 200-μl aliquots were placed in 4-ml clear glass tubes, and 200 μl of luminol (250 μg/ml in GBSS, freshly diluted from a stock of 25 mg/ml in dimethyl sulfoxide) was added. After a 10-min preincubation at 37°C the light emission was measured using a modified Locarte fluorimeter (light source "off," sensitivity controls maximum). A 200-μl aliquot of opsonized zymozan (1 mg/ml in GBSS, opsonized as described previously [7]) was added to each tube, and the mixture incubated at 37°C. Light emission was determined at 5-min intervals during the following 30 min.

Light output was shown to be proportional to cell density up to at least 2.5×10^7 cells/ml (i.e., 5×10^6 cells per assay). The instrument was calibrated daily using a standard light source of 25 μCi ^3H in scintillation fluid; this gave approximately 75% full-scale deflection (i.e., 750 empirical units) and did not vary by more than ± 5% during the course of the experiments.

Statistics

Nonparametric methods (Wilcoxon ranking test) were employed throughout for evaluation of levels of significance.

RESULTS

General

No differences were observed between monocytes from psoriatic patients and those from controls in absolute numbers, in morphology, or in their behavior during fractionation. Thus the percentage monocytes in cytocentrifuge preparations of MNL was 27 ± 5 for controls and 29 ± 6 for patients. Counterflow centrifugation of MNL suspensions gave monocyte fractions of 88 ± 3% purity, 79 ± 7% relative yield for controls and 90 ± 2% purity, 76 ± 14% relative yield for patients. Behavior during culture was again identical, both controls and patients showing similar morphologic changes and percentage cell losses during 2 weeks in culture.

Enzyme Studies: Fresh Monocytes

The levels of 5 enzymes in freshly isolated monocytes from 9 paired subjects plus 3 additional controls are illustrated in Fig 1. Nonparametric statistical analysis shows no significant difference between normal and psoriatic subjects at the level $p = 0.05$ for any enzyme; this holds true using either the paired subjects alone (2-tailed test) or analysis as independent groups of 12 and 9 subjects (1-tailed test).

Cyclic AMP levels

"Resting," adrenaline-stimulated, and PGE_1-stimulated cyclic AMP levels in freshly isolated monocytes from 9 paired subjects are shown in Table II. Again, no differences can be shown at the level $p = 0.05$. It should be noted that a total of 3 values has been omitted from Table II because of poor agreement between the duplicate samples; inclusion of these figures (not shown) does not alter this negative conclusion. Alternative statistical approaches (for example, analysis of percentage increases of cyclic AMP following stimulation) also failed to yield any significant difference between psoriatic and control subjects.

Chemiluminescence

Table III (column 2) summarizes the peak chemiluminescence, expressed in terms of the total MNL population, for 19 control–psoriatic pairs. In 13 of these pairs the percentage of monocytes was determined by counting stained cytocentrifuge preparations, and the data for these have been recalculated in terms of monocytes only (column 3). In neither case does the difference between control and psoriatic subjects reach the significance level of $p = 0.05$.

The time curves for light output following the addition of opsonized zymosan (based on monocyte counts and averaged for 13 pairs) are illustrated in Fig 2. It is seen that the time course of events is similar for controls and psoriatics.

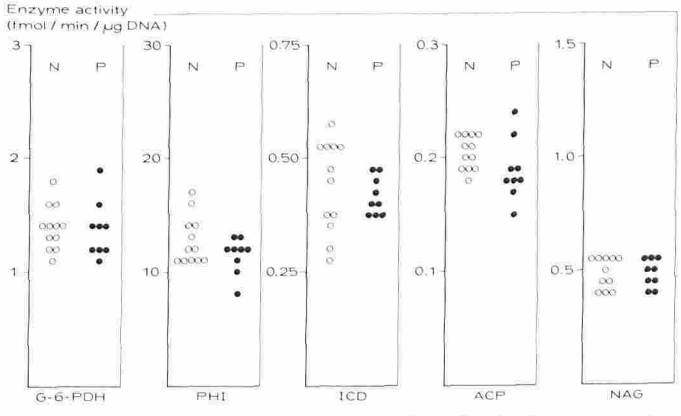

FIG 1. Activities of 5 enzymes in freshly isolated blood monocytes from normal controls (*open circles*) and psoriatic patients (*filled circles*).

TABLE II. *Cyclic AMP levels (pmol per 10^6 monocytes, mean ± SD) before and after hormonal stimulation*

Subjects	Cyclic AMP		
	Resting	+ Adrenaline	+ PGE_1
Controls	4.7 ± 4.0 (n=9)	10.7 ± 8.3 (n=9)	39.4 ± 26.9 (n=9)
Psoriatic	4.5 ± 4.9 (n=9)	16.0 ± 10.7 (n=7)	35.1 ± 18.3 (n=8)

Maturation in Culture

Four sets of cultures were set up from control subjects and two from psoriatic patients. Maturation was assessed by the levels of activity of the enzymes described for fresh monocytes, since it has already been established that certain of these (G-6-PDH, NAG, ACP) increase dramatically during the differentiation of monocytes to macrophages [14]. These results are summarized in Fig 3. Although the numbers are too small to permit statistical analysis, it is clear that the time course of maturation of the psoriatic cells (at least in terms of metabolic parameters) is indistinguishable from normal.

DISCUSSION

Cellular processes such as surface attachment, chemotaxis and phagocytosis are in general mediated by changes in cyclic nucleotide levels, which in turn trigger the alterations in metabolic activity necessary to support such functions. Thus it is unlikely that a "fault" could be manifest at one level of organization alone.

In the present investigation we have selected various parameters representing all three levels of organization; the individual measurements have been chosen on the basis of sensitivity (multiple assays using an acceptable blood sample), simplicity, and proven reliability. The agreement among independent experimental data at each level therefore greatly strengthens our overall conclusions, namely that we may exclude the concept of a primary abnormality in the psoriatic monocyte.

An overview of previous reports regarding metabolic activity, cyclic AMP levels, and chemiluminescence following phagocytosis is presented in Table IV; other studies of monocyte function in psoriasis are discussed by Krueger [1]. Clearly, an attempt must be made to explain the discrepancies between our present findings and the earlier literature. Three aspects will be considered here.

First, it must be emphasized that our patients were almost all in a quiescent phase of the disease, and that most had minimal lesions. Thus our findings do *not* rule out the possibility of secondary changes in patients with active or extensive psoriasis, and are not necessarily in conflict with studies employing such subjects [9]. In active psoriasis, as in other inflammatory processes (for example infections), monocytopoietic hyperproliferation occurs, probably reflecting increased monocyte consumption by the pathologic processes. This results inevitably in premature monocyte release from the bone marrow [15]; clearly, these relatively young monocytes may differ in many respects from the more mature cells.

Second, the possible effects of therapy must be considered.

TABLE III. *Peak chemiluminescence levels (arbitrary units, mean ± SD) following phagocytosis of opsonized zymosan*

Subjects	Chemiluminescence	
	per 10^6 MNL	per 10^6 Monocytes
Controls	266 ± 122 (n=19)	1054 ± 382 (n=13)
Psoriatic	313 ± 139 (n=19)	1143 ± 431 (n=13)

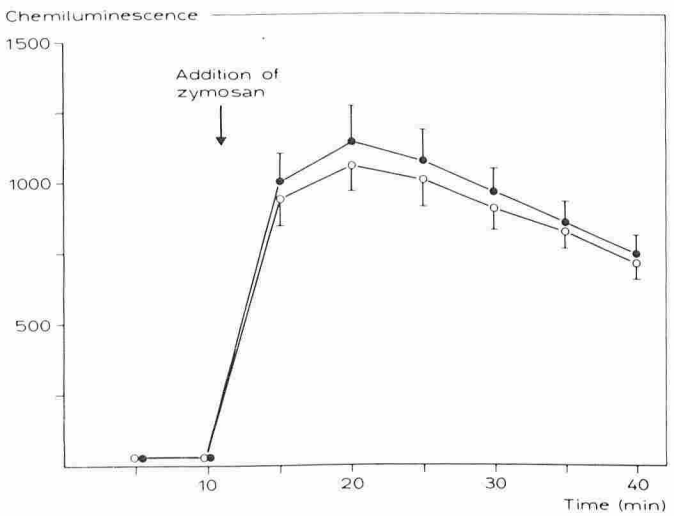

FIG 2. Time course of monocyte chemiluminescence. Opsonized zymosan was added immediately after the 10-min reading. *Open circles* represent healthy controls, *filled circles* psoriatic patients. Mean of 13 pairs ± SEM.

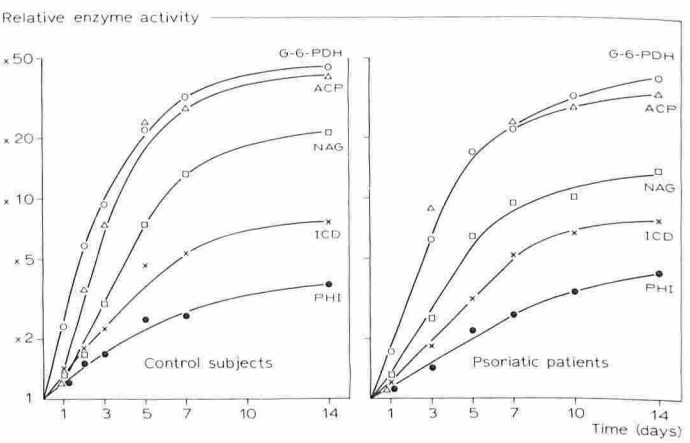

FIG 3. Changes in enzyme activity during cultivation of monocytes in vitro. The value on day 0 (freshly isolated monocytes) is designated as unity.

TABLE IV. *Summary of relevant literature*

Measurement	Ref	Cell type	Extent of disease	Therapy	Conclusions
Metabolic	8	MNL	Variable	None for 2 weeks	Increased NBT reduction, severity-related
	9	Mo	>25%	Topical steroids and/or tar	Resting HMPS activity normal; increased response to ADCC; lysosyme release during ADCC normal; superoxide production following PMA normal
Cyclic AMP	10	MNL	Variable	Topical	Resting and stimulated levels both slightly decreased
	11	Mo	Variable	Topical steroids and/or tar	Resting and stimulated levels normal
	9	Mo	>25%	Topical steroids and/or tar	Normal during ADCC
Chemiluminescence	12	—	—	—	Faster response and increased peak values following zymosan phagocytosis
	13	MNL	5–20%	Anthralin	Increased values following zymosan

Mo = monocytes; NBT = nitro blue tetrazolium; HMPS = hexose monophosphate shunt; ADCC = antibody-dependent cell-mediated cytotoxicity; PMA = phorbol myristate acetate.

It is extremely difficult, via a hospital system, to locate patients who really have received no therapy of any kind for a long period. Most workers therefore opt either for "topical therapy only" [9–11, 13] or withhold treatment for an arbitrary (usually short) period [8]. Here we have overcome this problem by locating psoriatic subjects via a nonmedical organization (Association of Psoriatic Patients), many of whose members are routinely untreated.

Finally, we would point out the extreme lability of certain blood leukocyte parameters, an observation that has received comparatively little attention in the literature. For example, cyclic AMP levels fall to 5–30% of their original values after 2 h [16]; similar declines in chemiluminescence have been observed [C. Figdor, personal communication]. Thus trivial variations in processing time may produce substantial variation in observed data, and any systemic differences among groups (such as transport of specimens from clinic to laboratory) may result in highly "significant" findings. In the present investigation, the use of time-matched patient–control pairs has circumvented this problem.

There has been considerable argument over the past decade as to whether the systemic immunologic disturbances in psoriasis are of etiologic significance or are secondary to the cutaneous lesions. Our present findings, although throwing no direct light on the pathogenesis of psoriasis, at least offer strong evidence in favor of the latter view.

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REFERENCES

1. Krueger GG: Psoriasis: current concepts of its etiology and pathogenesis, The Yearbook of Dermatology. Edited by RL Dobson, BH Thiers. Chicago/London, Year Book Publishers, 1981, pp 13–70
2. Mier PD, Gommans JM, Roelfzema H: On the etiology of psoriasis. Br J Dermatol 103:457–460, 1980
3. De Mulder PHM, Wessels JMC, Rosenbrand DA, Smeulders JBJM, Wagener DJT, Haanen C: Monocyte purification with counterflow centrifugation monitored by continuous flow-cytometry. J Immunol Methods 47:31–38, 1981
4. De Mulder PHM, Mier PD, De Pauw BE, Van Rennes H, Janssen JThP, Wagener DJT, Haanen C: Altered intracellular enzyme activity of monocytes and lymphocytes in Hodgkin's disease. Eur J Cancer Clin Oncol 19:333–337, 1983
5. Kapuscinsky J, Skoczylas B: Simple and rapid fluorometric method for DNA micro-assay. Anal Biochem 83:252–257, 1977
6. Mier PD, Van Rennes H, Van Erp PEJ, Roelfzema H: Cutaneous sialidase. J Invest Dermatol 78:267–269, 1982
7. Trush MA, Wilson ME, Van Dyke K: The generation of chemiluminescence (CL) by phagocytic cells. Methods Enzymol 57:462–494, 1978
8. Herlin T, Borregaard N, Kragballe K: On the mechanisms of enhanced monocyte and neutrophil cytotoxicity in severe psoriasis. J Invest Dermatol 79:104–108, 1982
9. Geerdink JPM, Bergers M, Van Erp PEJ, Gommans JM, Mier PD, Roelfzema H: Cyclic AMP is decreased in mononuclear leucocytes from psoriasis patients. Br J Dermatol 103:107–108, 1980
10. Herlin T, Kragballe K: Enhanced monocyte and neutrophil cytotoxicity and normal cyclic nucleotide levels in severe psoriasis. Br J Dermatol 105:405–414, 1981
11. Krueger GG, Jederberg WW: Monocyte function in patients with psoriasis as assessed by chemiluminescence and monokine production. Clin Res 27:137A, 1979
12. Schopf RE, Altmeyer P, Lemmel EM: Increased respiratory burst activity of monocytes and polymorphonuclear leucocytes in psoriasis. Br J Dermatol 107:505–510, 1982
13. De Mulder PHM, Van Rennes H, Mier PD, Van de Ven E, De Pauw BE, Haanen C: Characterization of monocyte maturation in adherent and non-adherent cultures and its application to study monocyte differentiation in Hodgkin's disease. Clin Exp Immunol: in press
14. Krueger GG, Jederberg WW, Ogden BE, Reese DL: Inflammatory and immune cell function in psoriasis: II. Monocyte function, lymphokine production. J Invest Dermatol 71:195–201, 1978
15. Meuret G, Schmitt E, Hagedorn M: Monocytopoiesis in chronic eczematous diseases, psoriasis vulgaris and mycosis fungoides. J Invest Dermatol 66:22–28, 1976
16. Goffstein BJ, Gordon LK, Wedner HJ, Atkinson JP: Cyclic AMP concentrations in human peripheral blood lymphocytes. Changes in association with cell purification. J Lab Clin Med 96:1002–1014, 1980